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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C12N 15/12, A61K 39/395, 48/00

A2

(11) International Publication Number: **WO 98/36061**

(43) International Publication Date: 20 August 1998 (20.08.98)

(21) International Application Number: PCT/GB98/00319

(22) International Filing Date: 13 February 1998 (13.02.98)

(30) Priority Data:
9702944.1 13 February 1997 (13.02.97) GB

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(81) Designated States: AU, CA, JP, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: REDUCING FIBROSIS AND/OR SCARRING BY INHIBITING INTERLEUKIN–6 RECEPTOR–MEDIATED ACTIVITY

(57) Abstract

The present application relates to the use of agents which inhibit Interleukin–6 receptor mediated activity for the treatment of wounds and/or fibrotic disorders such that fibrosis and/or scarring are reduced or prevented. Preferred agents for use in such treatments include Interleukin–6 neutralising antibodies, Interleukin–6 Receptor antagonists and inhibitors of Interleukin–6 Receptor signal transduction.

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REDUCING FIBROSIS AND/OR SCARRING BY INHIBITING INTERLEUKIN-6 RECEPTOR-MEDIATED ACTIVITY

The present invention relates to reducing fibrosis in the healing of wounds and other conditions in which fibrosis is a major mechanism of tissue repair or where excessive fibrosis leads to pathological derangement and malfunctioning of the tissue.

The term "wound" as used herein is exemplified but not limited to injuries to the skin. Other types of wound can involve damage, injury or trauma to an internal tissue or organ such as the lung, kidney, heart, gut, tendons or liver.

Wound healing in adult tissues is a complicated reparative process. The healing process begins with the recruitment of a variety of specialised cells to the site of the wound and involves extracellular matrix and basement membrane deposition, angiogenesis, selective protease activity and re-epithelialisation. An important component of the healing process in adult mammals is the stimulation of fibroblasts to generate the extracellular matrix. This extracellular matrix constitutes a major component of a connective tissue which develops to repair the wounded area and which may develop into a scar.

A scar is an abnormal morphological structure resulting from a previous injury or wound (e.g. an incision, excision or trauma). Scars are composed of a connective tissue which is predominately a matrix of collagen types 1 and 3 and fibronectin. The scar may consist of collagen fibres in an abnormal organisation (as seen in scars of the skin) or it may be an abnormal accumulation of connective tissue (as seen in scars of the central nervous system). Most scars consist of abnormally organised collagen and also excess collagen. In man, in the skin, scars may be depressed below the surface or elevated above the surface of the skin. Hypertrophic scars are a more severe form of normal scarring, are elevated above the normal surface of the skin and contain excessive collagen arranged in an abnormal pattern. A keloid is another form of pathological scarring which is not only elevated above the surface of the skin but also

extends beyond the boundaries of the original injury. In a keloid there is excessive connective tissue which is organised in an abnormal fashion predominately in whirls of collagenous tissue. There are genetic predispositions to forming both hypertrophic scars and keloids. They are particularly common in Africo-Carribean and Mongoloid races.

There are various medical situations for which it is desirable to promote the healing of wounds such that scar formation is regulated. Examples of such situations are scars of the skin where excessive scarring may be detrimental to tissue function and particularly when scar contracture occurs (for instance skin burns and wounds which impair flexibility of a joint). The reduction of scarring to the skin when cosmetic considerations are important is also highly desirable. In the skin, hypertrophic or keloid scars (particularly in Africo-Caribbean and Mongoloid races) can cause functional and cosmetic impairment and there is a need to prevent their occurrence. Scarring resulting from skin grafts in both donor sites and from the application of artificial skin can also be problematic and need to be minimised or prevented.

As well as scars of the skin, internal scarring or fibrosis can be highly detrimental and specific examples include:

(i) Within the central nervous system, glial scarring can prevent neuronal reconnection (e.g. following neuro-surgery or penetrating injuries of the brain).

(ii) Scarring in the eye can be detrimental. In the cornea, scarring can result in abnormal opacity and lead to problems with vision or even blindness. In the retina, scarring can cause buckling or retinal detachment and consequently blindness. Scarring following wound healing in operations to relieve pressure in glaucoma (e.g. glaucoma filtration surgery) results in the failure of the surgery whereby the aqueous humour fails to drain and hence the glaucoma returns.

(iii) Scarring in the heart (e.g. following surgery or myocardial infarction) can give rise to abnormal cardiac function.

(iv) Operations involving the abdomen or pelvis, often result in adhesion between viscera. For instance, adhesions between elements of the gut and the body wall may form and cause twisting in the bowel loop leading to ischaemia, gangrene and the necessity for emergency treatment (untreated they may even be fatal). Likewise, trauma or incisions to the guts can lead to scarring and scar contracture to strictures which cause occlusion of the lumen of the guts which again can be life threatening.

(v) Scarring in the pelvis in the region of the fallopian tubes can lead to infertility.

(vi) Scarring following injury to muscles can result in abnormal contraction and hence poor muscular function.

(vii) Scarring or fibrosis following injury to tendons and ligaments can result in serious loss of function.

Related to the above is the fact that there are a number of medical conditions known as fibrotic disorders in which excessive fibrosis leads to pathological derangement and malfunctioning of tissue. Fibrotic disorders are characterised by the accumulation of fibrous tissue (predominately collagens) in an abnormal fashion within the tissue. Accumulation of such fibrous tissues may result from a variety of disease processes. These diseases do not necessarily have to be caused by surgery, traumatic injury or wounding. Fibrotic disorders are usually chronic. Examples of fibrotic disorders include cirrhosis of the liver, liver fibrosis, glomerulonephritis, pulmonary fibrosis, scleroderma, myocardial fibrosis, fibrosis following myocardial infarction, central nervous system fibrosis following a stroke or neuro-degenerative disorders (e.g. Alzheimer's Disease), proliferative vitreoretinopathy (PVR) and arthritis. There is therefore also a need for medicaments which may be used for the treatment of such conditions by regulating (i.e. preventing, inhibiting or reversing) fibrosis / scarring in these fibrotic disorders.

There are however circumstances where the rate of healing is of primary importance and scar formation or fibrosis is only of secondary consideration. For example, it is often desirable to increase the rate of healing in the case of acute wounds (such as penetrative injuries, burns, nerve damage or even wounds resulting from elective surgery), chronic wounds (such as diabetic, venous and decubitus ulceration) or for generally healing compromised individuals (for example the elderly). In these examples, the wounds can severely influence quality of life or even result in death and therefore the rate of healing often needs to be increased as much as is clinically possible. When the rate of wound healing is increased using many conventional therapies, there is often an associated increase in scar formation but this may be of secondary importance compared to the need to increase the rate of healing. However it will be appreciated that it is desirable to be able to increase that rate of wound healing and to also limit scar formation or fibrosis in these circumstances.

It is known that, following wounding, there is an inflammatory and immune response which is at least partly mediated by a plurality of agents (e.g. chemotactic agents and growth factors) released at the wound site.

Thus, for example, it has been shown that Interleukin-6 (IL-6) increases rapidly upon initial tissue trauma, peaking within the first 12 hours (Mateo *et al.* Am J Physiol 266(6, Part 2): R1840-R1844), probably mediated in part by IL-1α expression (Goretsky *et al.* J Trauma Injury Infection and Critical Care. 40(6):894/899). Neutrophils, macrophages, lymphocytes, epithelial cells, keratinocytes and endothelial cells all produce IL-6 following wounding. Platelets are not a source of IL-6, so local levels are mainly due to release from the immediate influx of neutrophils.

WO-A-93/21771 (University of Utah) discloses that dehydroepiondrosterone (DHEA) congeners (e.g. DHEA and DHEA-S) may be used for the reduction ("down-regulation") of abnormally elevated IL-6 levels in a patient to restore normal IL-6 levels and/or alleviate one or more symptoms of a pathological condition associated

with elevated levels of IL-6. The DHEA may be used for example for the reduction of elevated IL-6 levels caused by trauma or an autoimmune disease.

It is also proposed in WO-A-93/21771 that DHEA may be used for the treatment of individuals who have abnormally elevated IL-6 levels and who are non responsive to growth factors such as PDGF, TGF-β and insulin. Such individuals may be suffering from disorders such as inhibition of wound healing, osteoporosis and diabetes respectively. This suggests that decreasing the level of IL-6 would (in persons who are non-responsive to growth factors) accelerate wound healing and increase fibrosis in scarring since both TGFβ (especially TGFβ1 which is the particular TGFβ which would be understood when TGFβ is referred to generically) and PDGF are pro-scarring, i.e. pro-fibrotic, growth factors.

WO-A-95/00103 discloses that antisense oligonucleotides to fibrogenic cytokines may be used to prevent or reduce fibrosis and/or scarring. More specifically WO-A-95/00103 speculates that an antisense oligonucleotide to IL-6 may have efficacy for preventing or reducing scar formation or fibrosis. However antisense oligonucleotides to IL-6 are not very versatile for use during wound healing and/or fibrosis because they cannot readily cross cell membranes to enter the cell nucleus (where they prevent IL-6 expression). At best these oligonucleotides are only able to enter a cell nucleus at the time of wounding when cells in the wound vicinity become transiently permeable such that the oligonulceotide may cross cellular membranes for entering into the cell nucleus. This means that for optimum effect the oligonucleotides are required to be delivered to the tissue before wounding so that they can be taken up into cells at the time of wounding. They are therefore most suitable for use in wounds incurred by elective surgery when the oligonucleotide may be administered in advance of wounding.

We have however now surprisingly found, and this forms the basis of the present invention, that agents which inhibit IL-6 receptor mediated activity are particularly effective for preventing or reducing fibrosis and/or scarring.

According to a first aspect of the present invention there is provided the use of an agent that inhibits Interleukin-6 receptor mediated activity for the manufacture of a medicament for preventing or reducing fibrosis and/or scarring.

According to a second aspect of the present invention there is provided a method of treating a patient to prevent or reduce fibrosis and/or scarring comprising administering to the patient a therapeutically effective amount of an agent that inhibits Interleukin-6 receptor mediated activity.

According to a third aspect of the present invention there is provided a composition for preventing or reducing fibrosis and/or scarring which comprises a therapeutically effective amount of an agent that inhibits Interleukin-6 receptor mediated activity and a pharmaceutically acceptable vehicle.

By "inhibits Interleukin-6 receptor mediated activity" we mean that the agent inhibits physiological effects mediated by Interleukin-6 present at the site of wounding, fibrosis or scarring. The inhibition of these physiological effects may be achieved by use of, for example, an agent which prevents or limits the interaction of IL-6 (present at the site of wounding, fibrosis or scarring) with its receptor, one which prevents activation of the IL-6 receptor by endogenous IL-6, one which reduces IL-6 receptor expression in cells at the site of wounding, fibrosis or scarring, one which increases IL-6 receptor degradation or one which prevents or inhibits post-receptor signal transduction mechanisms. Examples of suitable agents are given below.

Compared to the antisense oligonucleotides disclosed in WO-A-95/00103, the agents used according to the present invention are surprisingly effective for preventing

or reducing scar formation or fibrosis. A complex interacting cascade of cytokines (particularly the Interleukins) is thought to lead to the development of scars and/or fibrosis and it would be thought that the prevention of the expression of IL-6 (e.g. by the oligonucleotides disclosed by WO-A-95/00103) would be necessary to prevent the formation of this cascade and thereby prevent or reduce scarring and/or fibrosis. Agents which effect IL-6 receptor mediated activity (without modulating IL-6 expression) would have previously been expected to be less effective for preventing or reducing scarring and/or fibrosis because IL-6 would still be present at the wound site and would be able to recruit further cytokines into the cascade which leads to fibrosis and scarring. However we have found that the agents used according to the present invention are effective for preventing or reducing fibrosis and scarring despite the fact that IL-6 may be present in the wound or at the site of fibrosis.

Furthermore, agents which inhibit IL-6 receptor mediated activity according to the invention are not hindered by the permeability problems faced by antisense oligonucleotides which need to enter the cell nucleus. This is because many of the agents exert their effect by acting extracellularly (e.g. by combining with the IL-6 receptor which is a cell surface receptor).

Agents which inhibit Interleukin-6 receptor mediated activity are useful in situations or conditions where scarring needs to be prevented or reduced such as:

(i) where scars of the skin may be excessive and/or detrimental to tissue function and particularly when scar contracture occurs or may occur (for instance skin burns and wounds which impair flexibility of a joint and particularly scarring in children);

(ii) scarring to the skin when cosmetic considerations are important;

(iii) when hypertrophic or keloid scars (particularly in Africo-Caribbean and Mongoloid races) may occur which can cause functional and cosmetic impairment;

(iv) scarring resulting from skin grafts in both donor sites and from the application of artificial skin;

(v) scarring within the central nervous system (e.g. following neuro-surgery or penetrating injuries of the brain), for example glial scarring can prevent reconnection of severed neurons;

(vi) scarring in the eye and particularly of the cornea (scarring can result in abnormal opacity and lead to problems with vision or even blindness), in the retina (scarring can cause buckling or retinal detachment and consequently blindness) and scarring following wound healing in operations to relieve pressure in glaucoma (e.g. glaucoma filtration surgery) which can result in the failure of the surgery whereby the aqueous humour fails to drain and hence the glaucoma returns;

(vii) scarring in the heart (e.g. following surgery or myocardial infarction) which can give rise to abnormal cardiac function;

(viii) scarring of the gut such as may occur following operations involving the abdomen or pelvis that result in adhesion between viscera (adhesions between elements of the gut and the body wall can form and cause twisting in the bowel loop leading to ischaemia, gangrene and the necessity for emergency treatment -untreated they may even be fatal); likewise, trauma or incisions to the guts can lead to scarring and scar contracture or strictures which cause occlusion of the lumen of the guts which again can be life threatening;

(ix) scarring in the pelvis in the region of the fallopian tubes which can lead to infertility;

(x) scarring following injury to muscles which can result in abnormal contraction and hence poor muscular function;

(xi) scarring or fibrosis following injury to tendons and ligaments which can result in serious loss of function.

Inhibitors of IL-6 activity are also useful for treating fibrotic disorders such as cirrhosis of the liver, liver fibrosis, glomerulonephritis, pulmonary fibrosis, scleroderma, myocardial hibernation, fibrosis following myocardial infarction, central nervous system fibrosis following a stroke or neuro-degenerative disorders (e.g. Alzheimer's Disease), proliferative vitreoretinopathy (PVR) and arthritis.

A preferred use for inhibitors of IL-6 receptor mediated activity is in the prevention of inappropriate scar formation following a dermal wound.

It is an advantage of the present invention that the agents improve scar quality without any significant detriment to the rate of wound healing. Thus the agents may be used in circumstances where the rate of healing is of primary importance. For example, the agents may be used in the treatment of acute wounds (such as penetrative injuries, burns, nerve damage or even wounds resulting from elective surgery), chronic wounds (such as diabetic, venous and decubitus ulceration), for generally healing compromised individuals (for example the elderly) or for any other circumstance where the rate of healing needs to be increased as much as is clinically possible and an anti-scarring or anti-fibrotic agent would normally be contra-indicated. Thus for example an agent which inhibits IL-6 receptor mediated activity may be used with an agent which increases the rate of wound healing to also improve scar quality.

The agents are suited for use in subjects (human or animal) in which the amount of IL-6 associated with the wound is within the normal range associated with a healing wound.

Suitable inhibitors of IL-6 activity and thereby preferred compounds for use according to the invention include IL-6 Receptor antagonists (compounds which inhibit receptor activation by IL-6); compounds that disrupt signalling mediated by the activated IL-6 receptor (e.g. inhibitors of second messenger production, kinase inhibitors); enzymes that specifically degrade IL-6 (thus preventing IL-6 combining with its receptor), neutralising antibodies to IL-6 or its receptor, agents which increase IL-6 receptor degradation or sequestration from the cell surface, oligonucleotide aptmers which bind to and neutralise IL-6 or its receptor and molecules which bind to IL-6 and increase its clearance from a wound site.

Preferred agents are neutralising antibodies for IL-6 which prevent IL-6 from associating with its receptor. Such antibodies may be high affinity antibodies used at a high concentration because low affinity/ low concentrations of neutralising antibody are known to act as carrier protective agents and so potentiate the activity of IL-6 (Heremans et al. Eur. J. Immunol. 22 p2395-2401, 1992)). Examples of these preferred neutralising antibodies include the anti-human IL-6 antibody designated AF 206 NA and the anti-mouse IL-6 antibody designated AF 406 NA (both available from R & D Systems Inc., Minneapolis , USA). It will be appreciated that these antibodies are most suitable for inhibiting or preventing scarring and/or fibrosis in humans and mice respectively and that species specific antibodies may be easily developed for use in other animals.

Another preferred type of neutralising antibody is one raised against the IL-6 receptor. Such antibodies prevent IL-6 from activating the receptor. An example of this type of antibody is the anti-human antibody AF 227 NA (also available from R & D Systems Inc., Minneapolis , USA). Another example of such an antibody is a anti-GP130 antibody (GP130 being the second subunit of the IL-6 Receptor which also functions as a receptor subunit for LIF, OSM, CNTF and IL-11).

Other preferred compounds for use according to the invention are IL-6 Receptor antagonists and agents which prevent or inhibit post-receptor signal transduction mechanisms.

The agent for inhibiting the activity of IL-6 used according to the invention may be a protein or derivatives thereof (e.g analogues of IL-6 that act as IL-6 receptor antagonists). Alternatively agents that are non-proteins, but which nevertheless are pharmacologically active as inhibitors of IL-6 activity, may also be used.

The agent which is, or which is to be, administered to the patient may be one which *per se* inhibits Interleukin-6 (IL-6) receptor mediated activity or one which generates, or is converted to, an "active" agent within the body of the patient which in turn inhibits IL-6 receptor activity.

The medicaments and compositions of the invention may take a number of different forms depending, in particular on the manner in which the inhibitor of IL-6 activity is to be used. Thus, for example, the medicament or composition may be in the form of a liquid, ointment, cream, gel, hydrogel, powder or aerosol. It will be appreciated that the vehicle for the inhibitor of IL-6 activity should be one which is well tolerated by the patient and allows release of the active agent to the wound. Such a vehicle is preferably biodegradeable, biocompatable, bioresorbable, non-inflammatory, non-immunogenic. For instance,. the vehicle may comprise solutions or polymers of hyaluronic acid.

A medicament comprising an agent which inhibits IL-6 activity may be used in a number of ways. Thus, for example, a medicament in accordance with the first aspect of the invention may be applied in and/or around a wound of a patient to provide the desired reduction in fibrosis or scarring. Such a medicament may be provided on a sterile dressing or patch which may be used to cover or even pack a wound to be treated.

If the medicament is to be applied directly to an actual wound, trauma or injury, then the pharmaceutically acceptable vehicle will be one which does not cause an inflammatory response or is toxic to the tissue.

Topical application is a preferred means of administering agents which inhibit IL-6 activity to a subject (person or animal) in need of treatment.

It is possible to use medicaments in accordance with the invention as a prophylactic. For instance, prior to surgery (particularly elective surgery) it may be desirable to provide an agents which inhibits IL-6 activity for regulation of healing of the subsequently formed surgical wound so as to reduce scarring and/or treat a fibrotic disorder. In this case the vehicle of the composition will need to be one capable of delivering the agent to the target tissue. For example the vehicle may need to be suitable for carrying the agent across the keratinous layer of the skin. Examples of suitable vehicles for this purpose include dimethyl sulphoxide and acetic acid.

A further important application of agents which inhibit IL-6 activity relates to wound healing in the eye. For example, medicaments may be used to reduce or control scarring resulting from surgical operations on the eye, e.g. laser surgery on the cornea. In this case, the medicament of the invention may be in the form of eye drops. Alternatively the composition of the invention may be an injectable solution.

Medicaments in accordance with the invention may be used in a range of internal wound healing applications (in addition to that mentioned above for the eye). Thus for example, the composition may be formulated for inhalation (e.g. as an aerosol or spray) for use in wound healing of the lungs or for the prevention or treatment of fibrosis, adhesions and strictures in the lung. The medicaments may also be applied to internal organs of the abdomen and pelvis to prevent adhesions or strictures following surgery or arising from inflammatory conditions.

The medicaments and compositions may be administered by release from an implantable device (e.g. a biopolymer implant) Such release may be biological or externally triggered (e.g. by ultrasound).

It will be appreciated that the amount of an agent which inhibits IL-6 activity to be incorporated in a medicament and/or the amount of the agent to be applied to a wound site depends on a number of factors such as the biological activity and

bioavailability of the agent, which in turn depends on the mode of administration and the its physicochemical properties. Other factors include:

A) The half-life of the agent in the subject being treated.

B) The specific condition to be treated.

C) The age of the subject.

D) The sex of the subject

The frequency of administration will also be influenced by the above mentioned factors and particularly the half-life of the agent within the subject being treated.

Generally when used to treat existing wounds or fibrotic disorders it is usually advantageous to administer a medicament containing an agent which inhibits IL-6 receptor mediated activity as soon as the wound has occurred or the disorder has been diagnosed.

The medicaments are preferably applied within 48 hours post wounding and more preferably within 12 hours post wounding to realise the best anti-scarring results. It is particularly preferred that the medicament is applied at the time of, or shortly after (e.g. within 3 hours) post-wounding. However, fibrosis and scarring can develop over days or even weeks. Therefore the subject being treated may well benefit by commencing administration of an agent which inhibits IL-6 activity even if the medicament is first administered days or even weeks after the original wound occurred or the disorder developed (or was diagnosed).

Therapy with the agent should continue until the wound has healed to a clinicians satisfaction or, in the case of a fibrotic disorder, the risk or cause of abnormal fibrous tissue formation has been removed.

When used as a prophylactic (e.g. before surgery or when there is a risk of developing a fibrotic disorder) the medicament should be administered as soon as the risk of undesirable fibrosis has been recognised (as may be the case in people prone to develop keloid or hypertrophic scarring). For instance, a cream or ointment containing an IL-6 antagonist may be applied to a site on the skin of a subject where elective surgery is to be performed and reduced scarring of the subsequent wound is subsequently desired (e.g. surgery of the face or other cosmetically sensitive areas). In this case, the IL-6 antagonist may be applied during the preoperative preparation of the subject or it may even be desirable to apply the agent in the hours or days preceding the surgery (depending upon the health status and age of subject as well as the size of the wound to be formed).

Frequency of administration will depend upon the biological half-life of the agent used. Typically a cream or ointment containing a compound should be administered to a target tissue such that the concentration of the agent at the wound site or tissue affected by a fibrotic disorder is maintained at a level suitable for having a therapeutic effect. This may require administration daily or even several times daily.

Known procedures, such as those conventionally employed by the pharmaceutical industry (e.g. *in vivo* experimentation, clinical trials etc), may be used to establish specific formulations of medicaments and compositions and precise therapeutic regimes (such as daily doses of the compounds and the frequency of administration).

By way of example only a neutralising antibody may be administered to a wound as soon as the wound occurs. Such antibodies may be given twice daily and may be used for up to 5 days post-wounding.

Generally, medicaments and compositions in accordance with the invention will contain 0.001% to 10% by weight of the agent which inhibits IL-6 activity, preferably 0.0025% to 5% and more preferably 0.05% to 2.5%.

A suitable daily dose of an agent which inhibits IL-6 activity will depend upon the factors discussed above as well as upon the size of the wound, or extent of the fibrotic disorder, to be treated. Typically the amount of a compound required for the treatment of wounds or fibrotic disorders will be within the range of 1ng to 100g of the active compound/ 24 hours depending upon the size of the wound or extent of fibrosis amongst several other factors. By way of example, we have found that 0.3μg - 3mg/ 24 hour is a suitable quantity of a neutralising antibody for IL-6 (e.g. AF 206 NA for human wounds) to apply per linear centimetre of an incisional wound of the skin and more preferably 3μg - 300μg/ 24 hours.

A preferred means of using protein or peptide agents which inhibit Interleukin-6 receptor mediated activity is to deliver the agent to the wound, or other target tissue, by means of gene therapy. For instance, gene therapy could be used to increase expression of peptide antagonists of IL-6 receptors. Alternatively gene therapy may be used to express a neutralising antibody against IL-6 or its receptor. Therefore according to a fourth aspect of the present invention there is provided a delivery system for use in a gene therapy technique, said delivery system comprising a DNA molecule encoding for a protein which directly or indirectly prevents or reduces fibrosis and/or scarring by inhibiting Interleukin 6 receptor mediated activity, said DNA molecule being capable of being transcribed to lead to the expression of said protein.

According to a fifth aspect of the present invention there is provided the use of a delivery system as defined in the preceding paragraph for use in the manufacture of a medicament for preventing or reducing fibrosis and/or scarring.

According to a sixth aspect of the present invention there is provided a method of preventing or reducing fibrosis and/or scarring comprising administering to a patient in need of treatment a therapeutically effective amount of a delivery system as defined for the fourth aspect of the invention.

The delivery systems are highly suitable for achieving sustained levels of an active agent at a wound site or site of fibrosis over a longer period of time than is possible for most conventional delivery systems. Protein may be continuously expressed from cells at the wound site or site of fibrosis that have been transformed with the DNA molecule of the fourth aspect of the invention. Therefore, even if the protein has a very short half-life as an agent *in vivo*, therapeutically effective amounts of the protein may be continuously expressed from the treated tissue.

Furthermore, the delivery system of the invention may be used to provide the DNA molecule (and thereby the protein which is an active therapeutic agent) without the need to use conventional pharmaceutical vehicles such as those required in ointments or creams that are contacted with the wound. This is particularly beneficial as it can often be difficult to provide a satisfactory vehicle for a compound for use in wound healing (which are required to be non-inflammatory, biocompatible, bioresorbable and must not degrade or inactivate the active agent (in storage or in use)).

The delivery system is such that the DNA molecule is capable of being expressed (when the delivery system is administered to a patient) to produce a protein which directly or indirectly has activity for preventing or reducing fibrosis and/or scarring. By "directly" we mean that the product of gene expression *per se* has the required activity for regulating fibrosis or scarring. By "indirectly" we mean that the product of gene expression undergoes or mediates (e.g. as an enzyme) at least one further reaction to provide an agent effective for regulating fibrosis or scarring.

The DNA molecule may be contained within a suitable vector to form a recombinant vector. The vector may for example be a plasmid, cosmid or phage. Such recombinant vectors are highly useful in the delivery systems of the invention for transforming cells with the DNA molecule.

Recombinant vectors may also include other functional elements. For instance, recombinant vectors can be designed such that the vector will autonomously replicate in the nucleus of the cell. In this case, elements which induce DNA replication may be required in the recombinant vector. Alternatively the recombinant vector may be designed such that the vector and recombinant DNA molecule integrates into the genome of a cell. In this case DNA sequences which favour targeted integration (e.g. by homologous recombination) are desirable. Recombinant vectors may also have DNA coding for genes that may be used as selectable markers in the cloning process.

The recombinant vector may also further comprise a promoter or regulator to control expression of the gene as required.

The DNA molecule may (but not necessarily) be one which becomes incorporated in the DNA of cells of the subject being treated. Undifferentiated cells may be stably transformed leading to the production of genetically modified daughter cells (in which case regulation of expression in the subject may be required e.g. with specific transcription factors or gene activators). Alternatively, the delivery system may be designed to favour unstable or transient transformation of differentiated cells in the subject being treated. When this is the case, regulation of expression may be less important because expression of the DNA molecule will stop when the transformed cells die or stop expressing the protein (ideally when the fibrosis or scarring has been treated or prevented).

The delivery system may provide the DNA molecule to the subject without it being incorporated in a vector. For instance, the DNA molecule may be incorporated

within a liposome or virus particle. Alternatively the "naked" DNA molecule may be inserted into a subject's cells by a suitable means e.g. direct endocytotic uptake.

The DNA molecule may be transferred to the cells of a subject to be treated by transfection, infection, microinjection, cell fusion, protoplast fusion or ballistic bombardment. For example, transfer may be by ballistic transfection with coated gold particles, liposomes containing the DNA molecule, viral vectors (e.g. adenovirus) and means of providing direct DNA uptake (e.g. endocytosis) by application of plasmid DNA directly to the wounded area topically or by injection.

Whilst the above considerations mainly apply to wounds and fibrotic disorders of man it will be appreciated that wound healing, scarring and fibrosis can also be problematic in other animals (especially veterinary and domestic animals such as cattle, horses, dogs, cats etc). For instance tendon and ligament damage leading to scarring or fibrosis are a major reason for having to put down horses. The agents, medicaments, compositions and delivery systems discussed above are suitable for use in the healing of such animals.

The present invention will further be described in the following non-limiting Examples.

EXAMPLE 1

The inventors examined the influence of IL-6 on scarring during wound healing by assessing wound healing in IL-6 "knockout mice" (i.e. mice genetically engineered so as not to express IL-6) and normal "wild type" control mice. IL-6 knockout mice generated according to the experimental procedures disclosed by Kopf et al (Nature, 368 (6469) 239-342, 1994) were used in the present Example.

1.1. Methods

1.1.1 Treatments

A total of 55 mice were used (29 knockout and 26 wild type). The mice were anaesthetised using IP injection of Avertine, the dorsal surface shaved and two 1cm incisions made through the skin down to and including the panniculus carnosus muscle at specific anatomical positions. The wounds were left unsutured and the animals returned to individual cages. One group of animals was killed and the wounds harvested after 1 day(d), 3d, 5d, 7d, 14 and 70d post-wounding. Half the wound was fixed in formal saline and half embedded in OCT medium and frozen over liquid nitrogen. Photographic records were kept of the wounds at each time point, to enable comparison of microscopic and macroscopic results.

1.1.2 Histological Assessment.

Haematoxylin and Eosin (H&E) and Masson's Trichrome stains were used to determine the cellularity and connective tissue content of the wounds respectively (using 7μm wax sections of the harvested wounds).

1.1.3 Scoring of Scars 70 Days Post Wounding.

The histology slides were scored using a Visual Analogue Scale (VAS) consisting of a 10cm line where 0 represents normal skin and 10 a hypertrophic scar/keloid, and separately using a 0-5 rank scale, where 0 represents normal skin and 5 a hypertrophic scar/keloid and 3 is the score for a control scar.

1.2. Results

The IL-6 knockout mice had a decreased inflammatory reaction at 1 and 3 days. The wounds of the knockout mice contained fewer inflammatory cells at 1 and 3 days than control wounds. At 5 days the wounds of the IL-6 knockout mice had re-epithelialised and contained some inflammatory cells along with numerous fibroblasts. There was a greater amount of new collagen deposited within the knockout mice wounds compared to controls. At 7 and 14 days the wounds of the knockout mice contained a lot of newly synthesised collagen and numerous fibroblasts, suggesting fibroblast proliferation and migration at earlier stages had been increased. The scars at 40 and 70 days post-wounding contained more mature collagen fibres which were arranged in a more normal random orientation rather than aligned as observed in control wild type scars. Macroscopically, the scars were much finer and hardly visible in the knockout mice, compared to the control scars.

Scores for 70 Day Scars in Wild Type (WT) and Interleukin-6 Knockout (KO) Animals.

Slide	**Score (VAS)**	**Score (0-5)**
WT1	7.15	4
WT2	3.7	2
WT3	7.2	3
WT4	8.25	4
	Average=6.575	Average=3.25
KO1	5.1	3
KO2	1.4	1
KO3	3.45	2
KO4	2.1	1
	Average=3.01	Average=1.75

1.3. Summary

These results demonstrate that IL-6 deficient mice have superior wound healing such that there is decreased scar formation compared to control animals and show that agents which inhibit Interleukin-6 receptor mediated activity may be used to prevent or reduce scar formation. This is surprising because it is the opposite of the effect IL-6 would be expected to have in the light of the disclosure in WO-A-93/21771 (which teaches that a reduction in IL-6 activity causes an increase in the rate of wound healing and therefore suggests that inhibitors of IL-6 activity would be expected to increase scar formation).

The inventors further established that in IL-6 knockout mice there is not only significantly reduced scar formation following wounding but there is also only a slight retardation in the rate of wound healing.

EXAMPLE 2

A second set of experiments was performed using IL-6 knockout mice in which excisional wounds were made instead of incisional wounds. The same methods as used in Example 1 were employed except as indicated below.

A total of 11 mice were used (5 knockout and 6 wild type). Excisional wounds were created on the shaved dorsal surfaces of the mice. Two wounds of 5mm x 5mm were made at a distance of 2cm from the base of the skull and 1cm out from the spine. The wounds were harvested at 7 and 70 days post-wounding only. Photographic records of the wounds at 40 days were kept.

At 7d post-wounding, the wounds on the wild type mice were still very wide, had eschars and were not fully re-epithelialised. There was not much new collagen and few fibroblasts in the wounds. The excisional wounds from the IL-6 null mice

were quite cellular, some had re-epithelialised and most still had eschars, similar to the wild type wounds. At 70 days post-wounding, there were large visible scars on the backs of the control mice. The IL-6 knockout mice had mostly small, narrower scars which was also evident histologically. In some scars the collagen fibres were thicker, and not as aligned as in the scars from control mice. Macroscopically, it appeared that the square excisions had contracted in a linear manner in the IL-6 null mice, as the scars were narrow and linear at 70 days post-wounding.

These results further demonstrate that agents which inhibit Interleukin-6 receptor mediated activity may be used to prevent or reduce scar formation.

EXAMPLE 3

Experiments was performed using wild type mice (which express IL-6) to examine the effect of a neutralising antibody for IL-6 (an agent used according to the invention) on scar formation following incisional wounding. The same methods as used in Example 1 were employed except as indicated below.

3.1 Methods

2 incisional wounds were made on the back of a mouse as described in method 1.1.1. At the time of wounding, the first wound received 100µl (50µl down each wound margin) of 100µg/ml of AF 406 NA (an anti-mouse IL-6 antibody from R & D Systems Inc., Minneapolis, USA) and the second wound acted as a control and received 100µl of vehicle for the antibody only (Phosphate Buffered Saline - PBS). The first and second wounds received a further 100µl of antibody and vehicle respectively at both 12 hours and 24 hours post-wounding.

7 days post-wounding the wounds were harvested and histological assessment carried out as described in method 1.1.2.

3.2 Results

We found that after 7 days there was less fibronectin, inflammatory cells and blood vessels in the wound area of the IL-6 neutralising antibody treated wound compared to the control wound (PBS treated). This indicated that there is less fibrosis (and that scar formation is reduced) in the antibody treated wound than was observed in control wounds. This illustrates that agents used according to the invention are effective for reducing fibrosis and scarring.

CLAIMS

1. The use of an agent that inhibits Interleukin-6 receptor mediated activity for the manufacture of a medicament for preventing or reducing fibrosis and/or scarring.

2. The use according to claim 1 wherein the agent is one which prevents or limits the interaction of IL-6 with its receptor.

3. The use according to claim 2 wherein the agent is an anti-IL-6 neutralising antibody.

4. The use according to claim 2 wherein the agent is an anti-IL-6 Receptor neutralising antibody.

5. The use according to claim 2 wherein the agent is an IL-6 receptor antagonist.

6. The use according to claim 1 wherein the agent is one which prevents or inhibits post-receptor signal transduction mechanisms.

7. The use according to any one of claims 1 to 6 for the healing of wounds.

8. The use according to claim 7 for the healing of wounds where excessive scarring may be detrimental to tissue function.

9. The use according to claim 7 or 8 for wounds of the eye, nervous tissue, skin, internal organs, burns or acute wounds.

10. The use according to claim 7 or 8 for skin wounds to the face.

11. The use according to claim 7 or 8 for preventing or reducing the formation of hypertrophic scars or keloids.

12. The use according to any one of claims 1 to 6 for preventing or reducing connective tissue formation in fibrotic diseases.

13. The use according to claim 12, wherein the fibrotic disease is one of glomerulonephritis, liver cirrhosis, pulmonary fibrosis and scleroderma.

14. The use according to any one of claims 1 to 13 wherein the agent is provided in a composition which is a liquid, ointment, cream, gel, hydrogel, powder, aerosol or implantable device.

15. A method of treating a patient to prevent or reduce fibrosis and/or scarring comprising administering to the patient a therapeutically effective amount of an agent that inhibits Interleukin-6 receptor mediated activity.

16. A composition for preventing or reducing fibrosis and/or scarring which comprises a therapeutically effective amount of an agent that inhibits Interleukin-6 receptor mediated activity and a pharmaceutically acceptable vehicle.

17. A DNA molecule encoding for a protein which directly or indirectly prevents or reduces fibrosis and/or scarring by inhibiting Interleukin 6 receptor mediated activity, said DNA molecule being capable of being transcribed to lead to the expression of said protein.

18. The use of a delivery system according to claim 17 for use in the manufacture of a medicament for preventing or reducing fibrosis and/or scarring.

19. A method of preventing or reducing fibrosis and/or scarring comprising administering to a patient in need of treatment a therapeutically effective amount of a delivery system as defined in claim 17.

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International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12N 15/12, A61K 39/395, 48/00 — A3

(11) International Publication Number: WO 98/36061

(43) International Publication Date: 20 August 1998 (20.08.98)

(21) International Application Number: PCT/GB98/00319

(22) International Filing Date: 13 February 1998 (13.02.98)

(30) Priority Data:
9702944.1 13 February 1997 (13.02.97) GB

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(81) Designated States: AU, CA, JP, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(88) Date of publication of the international search report:
12 November 1998 (12.11.98)

(54) Title: REDUCING FIBROSIS AND/OR SCARRING BY INHIBITING INTERLEUKIN–6 RECEPTOR–MEDIATED ACTIVITY

(57) Abstract

The present application relates to the use of agents which inhibit Interleukin–6 receptor mediated activity for the treatment of wounds and/or fibrotic disorders such that fibrosis and/or scarring are reduced or prevented. Preferred agents for use in such treatments include Interleukin–6 neutralising antibodies, Interleukin–6 Receptor antagonists and inhibitors of Interleukin–6 Receptor signal transduction.

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INTERNATIONAL SEARCH REPORT

Inte ional Application No
PCT/GB 98/00319

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 A61K39/395 A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 12503 A (CHUGAI SEIYAKU KK) 2 May 1996 see abstract ---	1,2,4-6, 12-16
X	WO 96 18648 A (ISTITUTO DI RICERCHE DI BIOLOGIA MOLECOLARE P. ANGELETTI S.P.A.) 20 June 1996 see claims ---	1,2,5,6, 12-16
X	EP 0 617 126 A (AJINOMOTO CO., INC.) 28 September 1994 see examples see claims --- -/--	1-3,5, 12-19

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
24 August 1998	04/09/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Nooij, F

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Inte. ional Application No
PCT/GB 98/00319

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
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INTERNATIONAL SEARCH REPORT

...ernational application No.
PCT/GB 98/00319

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 15 and 19 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

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4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte ional Application No
PCT/GB 98/00319

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